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CRISPR-Engineered Anopheles Mosquitoes for Malaria Transmission Interruption: A Review of Preclinical and Early Field Evidence

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ABSTRACT

Malaria had been a leading global health challenge, with progress in control threatened by vector and parasite resistance. Conventional interventions such as insecticide-treated nets and artemisinin-based combination therapies have reduced burden but show diminishing effectiveness. Novel vector-focused strategies are therefore under urgent exploration. CRISPR-engineered gene drive mosquitoes represent a disruptive approach designed to either suppress vector populations or modify them to reduce Plasmodium transmission. The purpose of this review was to synthesize available preclinical and early field evidence on CRISPR-based Anopheles interventions, critically appraising their potential, limitations, and readiness for broader application. Literature was identified through structured searches of PubMed, Web of Science, and Scopus, focusing on studies published from 2010 through mid-2025, including laboratory, semi-field, and initial confined release trials. Preclinical findings indicate drive conversion efficiencies exceeding 90 percent in some constructs, though fitness costs and resistance allele emergence remain recurrent challenges. Contained population trials demonstrated stable transmission of modification constructs over multiple generations, but suppression drives showed variable persistence. Early field studies were limited, with only confined releases under stringent regulatory oversight, yet provide proof of feasibility for community engagement and monitoring frameworks. CRISPR gene drives in Anopheles show promise for malaria transmission interruption but require sustained evaluation of ecological safety, resistance management, and governance before widespread implementation.

Keywords: Malaria, Anopheles, CRISPR, Gene drive, Vector control

INTRODUCTION

Malaria continues to impose a major global burden, causing an estimated 249 million cases and 608,000 deaths worldwide in 2022 [1–3]. Most of the mortality occurs in sub-Saharan Africa, where more than 90 percent of deaths are among children under five years [4]. Despite large-scale distribution of insecticide-treated nets, indoor residual spraying, and effective case management with artemisinin-based therapies, the rate of decline in malaria incidence has slowed. Insecticide resistance is now established in over 80 malaria-endemic countries, while reports of artemisinin partial resistance in Plasmodium falciparum further threaten control gains [5]. These challenges underscore the urgent need for novel and sustainable vector control approaches that can overcome the limitations of conventional tools. Anopheles mosquitoes are the principal vectors of malaria, and genetic strategies targeting their populations offer a promising frontier [6]. CRISPR-Cas technology has transformed gene editing by enabling highly efficient, site-specific DNA modification [7]. When incorporated into gene drive systems, CRISPR can bias inheritance patterns, allowing engineered traits to spread rapidly through vector populations. Depending on construct design, such traits can suppress mosquito populations by impairing fertility or survival, or modify them to reduce vector competence for Plasmodium. The purpose of this review is to provide a critical synthesis of preclinical and early field evidence on CRISPR-engineered Anopheles mosquitoes for malaria transmission interruption. By evaluating laboratory data, contained trial results, ecological and biosafety considerations, and emerging regulatory frameworks, this review aims to clarify the state of the science and outline research priorities. The review is organized into the following sections: methods for literature selection; biology and genetics of Anopheles relevant to gene drives; CRISPR tools and system designs; preclinical laboratory evidence; early field

evaluations; ecological and biosafety considerations; regulatory and ethical frameworks; and challenges with future directions. The article concludes with a summary and recommendation for next steps.

METHODOLOGY

A structured literature search was conducted to identify studies on CRISPR-engineered Anopheles mosquitoes for malaria control. Databases searched included PubMed, Web of Science, and Scopus. The timeframe was January 2010 to June 2025, capturing the period during which CRISPR-based vector research expanded. Search terms combined "CRISPR," "gene drive," "Anopheles," "malaria," "transmission," "field trial," and related synonyms. Eligible sources included peer-reviewed articles, preprints with substantive experimental data, and authoritative reports from research consortia and regulatory agencies. Only studies describing preclinical laboratory experiments, contained semi-field evaluations, or early field engagement activities were included. Commentaries and modeling papers were consulted for context but not used as primary evidence. Data were extracted on drive design, editing efficiency, mosquito fitness, resistance allele formation, transmission dynamics, and ecological outcomes. A narrative synthesis was performed, emphasizing quantitative outcomes where available and highlighting evidence strengths, uncertainties, and methodological limitations.

Biology and Genetics of Anopheles Relevant to Gene Drive

Anopheles mosquitoes comprise more than 400 species, but transmission is dominated by Anopheles gambiae complex members, including Anopheles gambiae sensu stricto and Anopheles coluzzii [8, 9]. Their high anthropophily, indoor feeding, and rapid breeding cycles make them efficient vectors. Gene drive efficacy depends heavily on population structure and mating patterns. Anopheles species exhibit overlapping generations, high reproductive rates, and localized dispersal, features that facilitate rapid spread of engineered alleles under natural conditions [10, 11]. The genome of An. gambiae, sequenced in 2002, has been extensively annotated, revealing loci critical for reproduction, sex determination, and parasite interaction. Genes such as doublesex, involved in sex differentiation, and FREP1, linked to parasite invasion, have been prominent targets for editing [12]. Drive dynamics are also influenced by genetic diversity; natural polymorphisms at target loci can affect editing efficiency and drive stability. Understanding these baseline features is essential for rational design of CRISPR gene drive constructs intended for stable inheritance.

CRISPR Tools and Gene Drive Designs

CRISPR-Cas9 provides precise site-directed cleavage followed by homology-directed repair, enabling insertion of drive elements [13, 14]. Homing-based drives exploit this mechanism to copy themselves into homologous chromosomes, leading to inheritance rates exceeding Mendelian expectations. In laboratory studies, drive conversion efficiencies in Anopheles have often exceeded 90 percent under optimized conditions. Alternative architectures such as daisy-chain drives or split-drive systems are under development to provide self-limiting spread and enhanced biosafety. These approaches aim to balance efficacy with reversibility, acknowledging ecological and governance concerns surrounding unrestricted drives.

Two principal strategies dominate:

i. Population suppression drives target fertility or viability genes. Editing the female-specific exon of doublesex induces sterility, leading to population collapse in caged experiments within 7–11 generations [15].
ii. Population modification drives insert effector genes that impair parasite development. For example, constructs expressing single-chain antibodies against Plasmodium oocyst stages reduced transmission potential in experimental settings.

Preclinical Laboratory Evidence

Laboratory evaluations have provided critical data on performance, fitness, and resistance. Suppression drives targeting doublesex in An. gambiae achieved near-complete population elimination in cages within a year [16], with drive allele frequencies exceeding 95 percent before collapse. However, resistant alleles generated by non-homologous end joining accumulated in some populations, attenuating impact. Some constructs showed reduced male mating competitiveness by 20–30 percent, while others had minimal impact. In modification drives, effector gene expression sometimes reduced longevity by 10 percent, though not consistently across studies. Importantly, transmission blocking efficacy was measurable: lines expressing anti-Plasmodium antibodies achieved reductions in oocyst prevalence of up to 98 percent under laboratory infection challenges [17]. Resistance remains a recurring challenge. Studies report resistant allele formation rates of 1–10 percent per generation, with selection favoring functional resistant alleles that restore gene function. Strategies to counteract this include multiplexed guide RNAs, target site selection within highly conserved loci, and use of recoded rescue alleles. Preclinical evidence demonstrates strong proof of concept for both suppression and modification drives but highlights technical barriers requiring iterative optimization.

Early Field and Contained Field Trial Evidence

Field application remains in its infancy. Current evidence derives primarily from large-scale cage studies, semi-field trials in confined environments, and preparatory engagement activities in endemic countries. The Target Malaria

consortium has led several pivotal studies, releasing genetically sterile male An. gambiae under contained field conditions in Burkina Faso [18, 19]. These mosquitoes were not gene drive carriers but served to test infrastructure, biosafety, and community engagement mechanisms. Outcomes demonstrated the feasibility of maintaining regulatory compliance, ensuring containment, and achieving meaningful dialogue with local communities.

As of 2025, no CRISPR-based gene drive mosquitoes have been released in open environments. However, modeling studies calibrated with cage data predict that suppression drives could reduce local vector populations by more than 90 percent within 4–5 years of release, assuming high initial introduction frequency and limited resistance. The few confined semi-field studies have demonstrated sustained drive allele inheritance across multiple cohorts, though ecological realism remains limited. The main limitation of current evidence is scale. Laboratory and cage populations number in the thousands, whereas natural Anopheles populations reach millions, with complex ecological interactions. Translating contained results to field efficacy requires carefully staged evaluations under progressively less controlled settings, subject to rigorous regulatory oversight.

Ecological and Biosafety Considerations

Concerns surrounding ecological impacts are substantial. Gene drives designed for suppression risk reducing or eliminating local Anopheles populations, potentially altering predator–prey dynamics and ecosystem balance. While ecological redundancy among mosquito species is often cited, comprehensive evaluations of food web consequences are sparse. Non-target effects could arise through horizontal gene transfer, although empirical evidence remains limited. Reversibility strategies are actively researched. Split-drives and daisy drives provide self-limiting spread, and counter-drive constructs have been conceptualized to overwrite or inactivate prior drives. Monitoring frameworks incorporating genomic surveillance and ecological sampling are essential for early detection of unintended spread or resistance emergence. Biosafety protocols for current research emphasize physical containment, redundant genetic barriers, and staged progression from laboratory to confined field conditions [20, 21]. Risk assessments recommend incorporating molecular features that restrict drive propagation, ensuring opportunities for recall or termination in case of unforeseen outcomes.

Regulatory and Ethical Frameworks

Governance of gene drive technologies requires alignment across local, national, and international levels [22, 23]. Existing regulatory structures for genetically modified organisms provide a foundation but are not fully adapted to self-propagating constructs. Agencies in malaria-endemic countries have initiated guideline development, often in partnership with the World Health Organization [24]. Ethical considerations extend beyond biosafety to include social acceptability and justice. Community engagement is central, as demonstrated by extensive consultations in West African field sites. These activities highlight the importance of informed consent not only at individual but also community levels. Cross-border spread introduces additional complexity, as mosquitoes do not respect national boundaries, raising questions of regional governance and liability. Current policy discourse emphasizes adaptive regulation, transparency, and inclusivity. While frameworks are emerging, consensus on thresholds for release readiness, acceptable risk, and post-release monitoring remains incomplete. Broader societal deliberation, integrating scientific, ethical, and cultural perspectives, is necessary before any large-scale releases can proceed.

Challenges and Future Directions

Several interrelated challenges must be addressed before CRISPR gene drives can contribute meaningfully to malaria elimination.

i. **Technical barriers**: Resistance allele formation remains the primary obstacle, necessitating improved multiplexed designs and identification of highly conserved genomic targets.
ii. **Ecological uncertainties**: The long-term impact of suppressing or modifying Anopheles populations requires ecological studies extending beyond laboratory surrogates.
iii. **Safety and reversibility**: Development of molecular containment and counter-drive mechanisms is essential to mitigate risks.
iv. **Regulatory readiness**: Existing frameworks must evolve to balance innovation with precaution, ensuring community trust and international coordination.
v. **Equity and engagement**: Benefits must accrue to affected communities, with sustained engagement and respect for local perspectives guiding technology development [25].

Future research priorities include long-term cage and semi-field experiments under more natural conditions, integrated modeling with ecological data, development of reversibility mechanisms, and iterative refinement of engagement models. Multidisciplinary collaboration across molecular biology, ecology, ethics, and policy is indispensable.

CONCLUSION

CRISPR-engineered gene drive mosquitoes represent a transformative approach to malaria control, aiming either to suppress vector populations or modify them to reduce transmission competence. Preclinical evidence demonstrates that highly efficient drive conversion can be achieved, leading to population collapse in caged experiments or substantial reductions in parasite prevalence in modified lines. Nevertheless, resistance allele

formation, fitness costs, and ecological uncertainties present significant technical hurdles. Early field-related evidence is limited to contained releases and preparatory studies, which confirm feasibility of regulatory compliance, biosafety infrastructure, and community engagement, but do not yet provide efficacy data in natural transmission settings. Ecological and governance considerations remain paramount, as the potential for cross-border spread necessitates international coordination. The ethical imperative of inclusive community engagement is equally critical to legitimacy and sustainability. Overall, current findings support the feasibility of CRISPR gene drives as a powerful tool for malaria transmission interruption but highlight that substantial gaps remain. Addressing resistance, establishing reversibility mechanisms, and strengthening governance frameworks are prerequisites for any future open release. Future research should prioritize large-scale semi-field trials integrating ecological monitoring, reversibility safeguards, and robust community engagement before considering open-environment releases.

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